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Action spectrum of photoactivated phthalocyanine AlS_2Pc in tumor bearing mice

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The action spectrum of disulfonated aluminum phthalocyanine (AlS_2Pc) as a photosensitizer for photodynamic therapy (PDT) was evaluated *in vivo* from 640 to 710 nm on two murine tumors with different biological properties. Mice bearing MS-2 fibrosarcoma and mice bearing the pigmented tumor B_{16} melanoma were injected with 5 mg/kg of AlS_2Pc and irradiated with a light dose of 50 mW for 10 min for the MS-2 fibrosarcoma and of 100 mW for 10 min for the B_{16} melanoma. The action spectrum for both tumors presents a red shift with respect to the absorption spectrum of AlS_2Pc in saline. The effectiveness is limited for short wavelengths ($\leq$655 nm for MS-2 and $\leq$660 for B_{16}), whereas it increases at longer wavelengths and reaches its maximum at a peak (672 nm). For wavelengths beyond 672 nm the photodynamic activity remains up to 710 nm despite the significant decrease in absorption. The results obtained for both murine tumors seem therefore to indicate that an appreciable modification of the absorption spectrum takes place when AlS_2Pc is incorporated into tissues following systematic administration.

Key words: Action spectrum, photosensitization, phthalocyanines.

Introduction

Photodynamic therapy (PDT) is a new technique proposed for selective local destruction of malignant tumor tissue.[1] This approach is based on the systemic administration of photosensitizing agents. During the photodynamic reaction, the photosensitizer is excited by a specific wavelength of light. The excited photosensitizer, subsequently, transfers its energy to a molecular substrate, such as oxygen, that causes irreversible oxidation of some essential cellular components.[2] In the literature its applicability to the treatment of experimental[3] and human tumors[4] is already known. Phase III trials are currently in progress for malignancies of the esophagus, bronchus and bladder.[5,6] Unfortunately, Photofrin II (the photosensitizer used in clinical PDT) exhibits chemical, pharmacological and photophysical properties which are not optimal for PDT.[7] Photofrin II is a mixture of monomeric and aggregated porphyrin molecules that possess weak absorption at a wavelength greater than 600 nm (red light is most commonly used in PDT to permit deep light penetration into mammalian tissues).[8] This drug is also retained by normal tissues, like skin, for extended time intervals following administration.[9] Such a retention leads to phototoxic reactions arising from exposure to sunlight or even bright artificial light.[10] Because of all these drawbacks, considerable effort is being devoted to developing new and more efficient tumor localizing photosensitizers with greater extinction coefficients of longer wavelength.

Recently, some of this interest has been focused on the sulfonated phthalocyanines (SPcs). Some SPcs are accumulated by tumors, are relatively easy to synthetize, water soluble, non-toxic and, in contrast to porphyrins, they strongly absorb clinically useful red light (600–700 nm).[11] Usually, they are composed of a mixture of mono-, di-, tri- and tetra-sulfonated analogs and the degree of sulfonation affects various potentially useful qualities: water solubility, cell penetration and localization, degree of aggregation and generation of singlet oxygen.[12] Several phthalocyanines were also proved to be efficient tumor photosensitizers.[13]

In our laboratory we are studying the cytocidal activity of photoactivated aluminum disulfonated phthalocyanines (AlS_2Pc) in solid experimental tumors. We have already reported that AlS_2Pc,

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when irradiated at 670 nm, where the absorption is maximum, shows a strong antitumor effect on two murine tumors with different biological properties, the MS-2 fibrosarcoma and the B_{16} melanoma.[14] On the basis of the results obtained, the aim of the study was to evaluate the action spectrum of AlS_2Pc *in vivo* in order to compare the tumor response as a function of the excitation wavelength with respect to the absorption spectrum. The experiments were performed on two murine tumors to evaluate the possible influence of different invasiveness and tissue pigmentation.

Material and methods

Animals and tumors

BALB/c, C_{57}Bl/6 and hybrid DBA/2 × BALB/c male mice, 8–10 weeks old, obtained from Charles River, Calco, Italy, were used and are hereafter called BALB, C_{57} and $CD2F_1$, respectively. MS-2 fibrosarcoma originally induced by the Moloney murine sarcoma virus and B_{16} melanoma of spontaneous origin were maintained in the laboratory by weekly i.m. passage of tumor cell homogenate into the right hind leg of BALB and C_{57} mice, respectively.

For the experiments, tumors from mice were removed under sterile conditions. The cell suspension was obtained by Potter homogenization, counted under optical light microscopy and injected intradermally (i.d.; 10^6 cell/mouse). Treatment started when the tumor mass measured approximately 1 cm in diameter. The animals were injected i.v. with the drug and irradiated with the laser 24 h later. A single light irradiation was done in all experiments.

Chemicals

AlS_2Pc was kindly provided by Professor T.G. Truscott, Department of Chemistry, University of Keele (UK). The drug was dissolved in physiological solution at a concentration of 5 mg/cm^3.

Irradiation sources

The argon-pumped dye laser was tuned at 640, 655, 660, 665 and 672 nm, while the CW Argon-pumped titanium–sapphire laser was tuned at 680, 685, 690, 695 and 710 nm. The listed wavelengths were selected to cover the whole absorption peak of AlS_2Pc in the visible range. In particular, 672 nm exactly corresponds to its absorption maximum.

A beam splitter in the laser path provided two beams of equal power. Both were coupled into 400 nm core optical fibers. The fiber tips were cut and their outputs used to irradiate two mice simultaneously. The animals were caged in a plastic box with a large hole (approximately 3 cm diameter) corresponding to the tumor area. The distance from the fiber tip to the tumor surface was adjusted so that the irradiated area had a diameter of approximately 1.5 cm. No significant increase of the tumor temperature was detected during the irradiation time. The temperature was measured by a needle thermocouple. The laser power was checked before and after each irradiation by a calibrated thermopile and its fluctuation were less than 10%.

Statistical analysis

The significance of differences in survival time was compared using the Mann–Whitney *U*-test.

Results and discussion

To single out the optimum irradiation wavelength for AlS_2Pc, its action spectrum was evaluated on mice bearing the fibrosarcoma MS-2 (Table 1). According to the results obtained in other experiments[14] the animals were injected with 5 mg/kg of AlS_2Pc and 24 h later were irradiated. The light dose was 50 mW/cm^2 for 10 min of exposure. This value was chosen to be half of the dose previously used[14] in order to avoid any artificial flattering of the measured action spectrum and to allow an estimate of the therapeutic efficacy as a function of wavelength.

The therapeutical effectiveness follows the absorption spectrum (Figure 1) from 640 nm up to the maximum (672 nm). In fact, the median survival time (MST) does not significantly differ from that of the control mice at short wavelengths (655 nm), whereas it increases at longer wavelengths and reaches its maximum at the peak (Table 1).

For wavelengths beyond 672 nm, a different behavior is observed in the action spectrum. The antitumor response remains appreciably high up to 710 nm despite the significant decrease in the absorption. It is worth noting that at 710 nm the absorption is remarkably lower than at 655 nm.

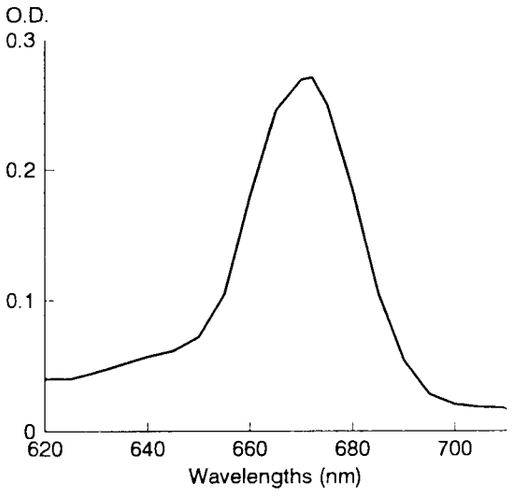

Figure 1. Absorption spectrum of 4 μM AlS_2Pc in physiological solution.

Moreover, a previous experiment performed with laser light at 700 nm did not show any effect.[15]

A second set of experiments was performed to measure the action spectrum of AlS_2Pc on mice challenged with the highly metastatic pigmented tumor B_{16} melanoma (Table 2). The murine melanoma was also adopted in our studies because of its peculiar biological properties, which make light transmission difficult.

The animals were injected with 5 mg/kg of AlS_2Pc and 24 h later were irradiated with a light dose of 100 mW/cm^2 for 10 min of exposure. These values were adopted according to the therapeutic protocol previously obtained[14] and they were not changed since, for this type of tumor, no complete cure of the animals was ever achieved.

Table 1. Action spectrum of photoactivated AlS_2Pc on MS-2 fibrosarcoma

Treatment drug[a]	Laser[b] (irradiation wavelength) (nm)	MST		Dead animals/total
—	—	56	(41–67)[c]	8/8
AlS_2Pc	640	65	(60–72)	8/8
AlS_2Pc	655	62	(59–70)	8/8
AlS_2Pc	660	70*	(67–75)	8/8
AlS_2Pc	665	82.5**	(75–87)	8/8
AlS_2Pc	672	—	(87–92)	3/8
AlS_2Pc	680	—	(85–91)	4/8
AlS_2Pc	685	—	(84–90)	4/8
AlS_2Pc	690	—	(85–93)	4/8
AlS_2Pc	695	86**	(83–91)	6/8
AlS_2Pc	710	—	(87–94)	4/8

CD2F$_1$ mice challenged i.d. with 10^6 MS-2 fibrosarcoma.
[a] AlS_2Pc, 5 mg/kg i.v.
[b] Laser, 50 mW/cm^2 for 10 min exposure.
[c] Number in parenthesis, range (days) of survival time.
* $p < 0.05$ by Mann–Whitney *U*-test vs. control group.
** $p < 0.001$ by Mann–Whitney *U*-test vs. control group.

Table 2. Action spectrum of photoactivated AlS_2Pc on B_{16} melanoma

Treatment drug	Laser[b] (irradiation wavelength) (nm)	MST		Dead animals/total
—	—	28	(25–32)[c]	8/8
AlS_2Pc	640	30	(27–34)	8/8
AlS_2Pc	655	32	(29–34)	8/8
AlS_2Pc	660	36	(32–40)	8/8
AlS_2Pc	665	38*	(35–42)	8/8
AlS_2Pc	672	56**	(50–61)	8/8
AlS_2Pc	680	54**	(50–62)	8/8
AlS_2Pc	685	57**	(50–65)	8/8
AlS_2Pc	690	53**	(48–61)	8/8
AlS_2Pc	695	55**	(49–63)	8/8
AlS_2Pc	710	53**	(47–63)	8/8

C57 mice challenged i.d. with 10^6 B_{16} melanoma.
[a] AlS_2Pc, 5 mg/kg i.v.
[b] Laser, 100 mW/cm for 10 min of exposure.
[c] Number in parenthesis, range (days) of survival time.
* $p < 0.05$ by Mann–Whitney *U*-test vs. control group.
** $p < 0.001$ by Mann–Whitney *U*-test vs. control group.

The results are in agreement with those obtained with the MS-2 fibrosarcoma. The effectiveness on the blue side is reduced with respect to the MS-2 model. Actually, taking into account the pigmentation of the B_{16} melanoma, reduced light penetration is expected in this spectral region. The good tumor response for wavelengths longer than the peak value, which is similar for both tumor models, cannot be explained simply in terms of the light penetration depth (particularly for excitation at 710 nm). In fact, on the basis of the values reported in the literature for various tissues,[16,17] the difference in average light penetration depth between 665 and 710 nm is less than a factor of 2. However, the difference in absorbance of AlS_2Pc in saline at the same wavelengths is about a factor of 8. Similar observations were obtained in organic solvents (e.g. methanol and benzene), in calf serum and albumin. Although no clear experimental evidence allows us to justify this behavior, the results obtained seem to suggest that an appreciable modification of the absorption spectrum takes place when AlS_2Pc is incorporated into tissues following systemic administration. In fact, since only the wavelength was varied during the experiment, the light absorption

must play a major role and, to our knowledge, no endogenous chromophores selectively absorb in this region.

Moreover, broadening of the ZnPc absorption spectrum has been observed when the phthalocyanine is incorporated in liposomes. (Jori, unpublished observation). Further experiments are in progress to elucidate these aspects and, in particular, to measure the absorption spectrum of AlS_2Pc *in vivo*.

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